IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	DOYLE ET AL.	Examiner:	UNKNOWN
Serial No.:	09/750,857	Group Art Unit:	2872
Filed:	DECEMBER 29, 2000	Docket No.:	12818.1USU1
Title:	METHODS AND COMPOSITIONS FOR INHIBITING ADHESION BY MICROORGANISMS		

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited in the United States Postal Service, as first class mail, with sufficient postage, in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231 on June 15, 2001.

By: Kristie A. Wacek
Name: Kristine A. Wacek

PRELIMINARY AMENDMENT

Box Missing Parts
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination please amend the above-identified patent application as follows.

In the Specification

Please replace the paragraph beginning at page 51, line 20, with the following rewritten paragraph:

--Treatment of bacteria with polyphenol oxidase at a concentration of 71 units/ml resulted in little reduction of adhesion (6%), whereas treatment with polyphenol oxidase at concentrations of 141 units/ml and 282 units/ml resulted in greater decreases in adhesion (60% and 44%, respectively) (Fig. 5). The fact that the highest polyphenol oxidase concentration was less effective than the 141 units/ml was seen consistently for polyphenol oxidase treatment of various adhesins. Although not limiting to the present invention, this finding may be due to the formation of Schiff's bases with surrounding proteins which at high concentrations that could include proteins on the host cell membrane.--

Please replace the paragraph beginning at page 51, line 28 to page 52, line 2, with the following rewritten paragraph:

--Treatment of type 1-fimbriated *E. coli* with asparaginase resulted in more consistent results than those obtained after treatment with polyphenol oxidase. Concentrations of asparaginase < 2 units/ml resulted in limited decreases in adhesion (13%); however, concentrations > 2 units/ml greatly decreased adhesion (85-90%) to UECs (Fig. 6).--

Please replace the paragraph beginning at page 52, line 3, with the following rewritten paragraph:

--Subjecting the bacteria to sequential enzyme treatments, either polyphenol oxidase followed by asparaginase or vice versa, did not have as great as an effect on reducing bacterial adhesion to UECs as the enzymes did singly. polyphenol oxidase, 141 units/ml, followed by asparaginase, 10 units/ml, resulted in only a 25% decrease in adhesion, while asparaginase, 10 units/ml, followed by polyphenol oxidase, 141 units/ml, gave a 45% decrease in adhesion. Even though these treatments did produce a reduction in adhesion, polyphenol oxidase and asparaginase singly provided much better prevention of adhesion, 60% and 90% respectively (Fig. 7).--

Please replace the paragraph beginning at page 52, line 11, with the following rewritten paragraph:

--To probe the enzymatic site of action, type 1-fimbriated *E. coli* were incubated with four-methylumbelliferyl α-D-mannopyranoside (MUMB, 50 mM) so as to protect the binding site followed by treatment with either polyphenol oxidase (141 units/ml) or asparaginase (10 units/ml). These treatments resulted in a 25% and 50% reduction in adhesion, respectively. Bacteria were incubated with the mannopyranoside in varied concentrations (10 mM, 50 mM, or 200 mM) then treated with polyphenol oxidase at a concentration of 141 units/ml to observe for a dose dependent effect. The percent of decrease of adhesion remained virtually unchanged (~ 30%) for each concentration of the mannopyranoside tested; therefore, 50 mM was used for further assays. The bacteria were incubated with the mannopyranoside (50 mM) followed by polyphenol oxidase (141 units/ml) or asparaginase (10 units/ml), resulting in a 25% decrease in adhesion and 40% increase in adhesion to UECs respectively (Fig. 8).--

Please replace the paragraph beginning at page 52, line 25, with the following rewritten paragraph:

--Treatment of bacteria with polyphenol oxidase at a concentration of 71 units/ml consistently resulted in a 40% reduction in adhesion. Treatment with polyphenol oxidase at concentrations of 141 units/ml and 282 units/ml averaged decreases in adhesion of 30% and 55%, respectively (Fig. 9). Treatment of P-fimbriated *E. coli* with increasing concentrations of asparaginase (2.5, 5, and 25 units/ml) resulted in 45, 55, and 85% decreases in adhesion respectively (Fig. 10).--

Please replace the paragraph beginning at page 53, line 1, with the following rewritten paragraph:

--Subjecting P-fimbriated *E. coli* to sequential enzyme treatments, either polyphenol oxidase followed by asparaginase or vice versa, had varying effects on reducing bacterial adhesion to UECs. polyphenol oxidase, 141 units/ml, followed by asparaginase, 10 units/ml, resulted in a 55% decrease in adhesion, while asparaginase, 10 units/ml, followed by polyphenol oxidase, 141 units/ml, resulted in no decrease from control adhesion (Fig. 11).--

Please replace the paragraph beginning at page 53, line 6, with the following rewritten paragraph:

--To probe the enzymatic site of action, P-fimbriated *E. coli* were incubated with globoside to protect the binding site followed by treatment with either polyphenol oxidase (282 units/ml) or asparaginase (5 units/ml). These treatments resulted in adhesion to UECs that was nearly the same as that of untreated bacteria (Fig. 12).--

Please replace the paragraph beginning at page 53, line 27, with the following rewritten paragraph:

--Figure 13 depicts the relative adhesion of *S. pyogenes* to buccal epithelial cells as measured using flow cytometry.--

REMARKS

Applicants respectfully request the Examiner to enter the above amendment. No new matter is introduced in the amendment. The changes made to the specification by the current amendment are attached hereto in a page entitled, "Version with Markings to Show Changes Made."

If the Examiner believes a telephone conference would advance the prosecution of this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

Respectfully submitted,

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PATENT TRADEMARK OFFICE

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

Paragraph beginning at page 51, line 20 has been amended as follows:

Treatment of bacteria with polyphenol oxidase at a concentration of 71 units/ml resulted in little reduction of adhesion (6%), whereas treatment with polyphenol oxidase at concentrations of 141 units/ml and 282 units/ml resulted in greater decreases in adhesion (60% and 44%, respectively) (Fig. 5 [1]). The fact that the highest polyphenol oxidase concentration was less effective than the 141 units/ml was seen consistently for polyphenol oxidase treatment of various adhesins. Although not limiting to the present invention, this finding may be due to the formation of Schiff's bases with surrounding proteins which at high concentrations that could include proteins on the host cell membrane.

Paragraph beginning at page 51, line 28 to page 52, line 2 has been amended as follows:

Treatment of type 1-fimbriated *E. coli* with asparaginase resulted in more consistent results than those obtained after treatment with polyphenol oxidase. Concentrations of asparaginase < 2 units/ml resulted in limited decreases in adhesion (13%); however, concentrations > 2 units/ml greatly decreased adhesion (85-90%) to UECs (Fig. 6 [2]).

Paragraph beginning at page 52, line 3 has been amended as follows:

Subjecting the bacteria to sequential enzyme treatments, either polyphenol oxidase followed by asparaginase or vice versa, did not have as great as an effect on reducing bacterial adhesion to UECs as the enzymes did singly. polyphenol oxidase, 141 units/ml, followed by asparaginase, 10 units/ml, resulted in only a 25% decrease in adhesion, while asparaginase, 10 units/ml, followed by polyphenol oxidase, 141 units/ml, gave a 45% decrease in adhesion. Even though these treatments did produce a reduction in adhesion, polyphenol oxidase and asparaginase singly provided much better prevention of adhesion, 60% and 90% respectively (Fig. 7 [3]).--

Paragraph beginning at page 52, line 11 has been amended as follows:

To probe the enzymatic site of action, type 1-fimbriated *E. coli* were incubated with four-methylumbelliferyl α-D-mannopyranoside (MUMB, 50 mM) so as to protect the binding site followed by treatment with either polyphenol oxidase (141 units/ml) or asparaginase (10 units/ml). These treatments resulted in a 25% and 50% reduction in adhesion, respectively. Bacteria were incubated with the mannopyranoside in varied concentrations (10 mM, 50 mM, or 200 mM) then treated with polyphenol oxidase at a concentration of 141 units/ml to observe for a dose dependent effect. The percent of decrease of adhesion remained virtually unchanged (~ 30%) for each concentration of the mannopyranoside tested; therefore, 50 mM was used for further assays. The bacteria were incubated with the mannopyranoside (50 mM) followed by polyphenol oxidase (141 units/ml) or asparaginase (10 units/ml), resulting in a 25% decrease in adhesion and 40% increase in adhesion to UECs respectively (Fig. 8 [4]).

Paragraph beginning at page 52, line 25 has been amended as follows:

Treatment of bacteria with polyphenol oxidase at a concentration of 71 units/ml consistently resulted in a 40% reduction in adhesion. Treatment with polyphenol oxidase at concentrations of 141 units/ml and 282 units/ml averaged decreases in adhesion of 30% and 55%, respectively (Fig. 9 [5]). Treatment of P-fimbriated *E. coli* with increasing concentrations of asparaginase (2.5, 5, and 25 units/ml) resulted in 45, 55, and 85% decreases in adhesion respectively (Fig. 10 [6]).

Paragraph beginning at page 53, line 1 has been amended as follows:

Subjecting P-fimbriated *E. coli* to sequential enzyme treatments, either polyphenol oxidase followed by asparaginase or vice versa, had varying effects on reducing bacterial adhesion to UECs. polyphenol oxidase, 141 units/ml, followed by asparaginase, 10 units/ml, resulted in a 55% decrease in adhesion, while asparaginase, 10 units/ml, followed by polyphenol oxidase, 141 units/ml, resulted in no decrease from control adhesion (Fig. 11 [7]).

Paragraph beginning at page 53, line 6 has been amended as follows:

To probe the enzymatic site of action, P-fimbriated *E. coli* were incubated with globoside to protect the binding site followed by treatment with either polyphenol oxidase (282 units/ml) or

asparaginase (5 units/ml). These treatments resulted in adhesion to UECs that was nearly the same as that of untreated bacteria (Fig. 12 [8])

Paragraph beginning at page 53, line 27 has been amended as follows:

Figure 13 [9] depicts the relative adhesion of *S. pyogenes* to buccal epithelial cells as measured using flow cytometry.